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Review

Dissolution of materials in artificial skin surface film liquids ☆

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Abstract

The dissolution of chemical constituents from jewelry, textiles, cosmetics, drugs, industrial chemicals, and particles in direct and prolonged contact with human skin is often assessed *in vitro* using artificial skin surface film liquids (SSFL). To provide meaningful results, the composition of artificial SSFL should accurately mimic human sweat and sebum, and the conditions of the *in vitro* test system should accurately reflect *in vivo* skin conditions. We summarized the reported composition of human SSFL and compared it to 45 different formulations of artificial sweat and 18 formulations of artificial sebum (studies published from 1940 to 2005). Conditions of *in vitro* dissolution test systems were reviewed and compared to *in vivo* skin conditions. The concentrations of individual constituents and pH of artificial sweat and concentrations of artificial sebum constituents are not always within ranges reported for human SSFL. Nearly all artificial SSFL lack many of the constituents in human SSFL. To develop a comprehensive model SSFL, we propose a standard SSFL, modified from the two best published sweat and sebum formulations. Little is known concerning the influence of test system conditions on dissolution, including SSFL temperature, container material composition, agitation, and physicochemical properties of the test article on dissolution. Thus, both a need and an opportunity exist for standardizing the composition of artificial SSFL and *in vitro* dissolution test methodologies. To standardize *in vitro* dissolution test systems, we recommend: maintaining artificial SSFL at a biologically relevant temperature appropriate to the human activity being modeled, carefully selecting test and sample storage containers to avoid bias in dissolution measurements, accounting for friction between a test article and skin in a biologically plausible manner, and physicochemical characterization of the test article or material to better understand mechanisms of dissolution and potential mechanisms of toxic action of dissolved material. More accurate modeling and better understanding of chemical dissolution from articles in contact with the skin will ultimately improve risk decision making, thereby protecting even the most susceptible persons from adverse health effects resulting from skin exposure.

Keywords: Dissolution; Skin; Artificial sebum; Human sebum; Artificial sweat; Human sweat; Skin surface film liquids

Contents

☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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1. Introduction

Chemicals that leach from jewelry, fasteners, textiles, cosmetics, industrial chemicals, and particles in contact with human skin surface film liquids (SSFL, a mixture of sweat and sebum) may cause adverse health effects such as irritation or contact dermatitis. In contrast, constituents that leach from topical application pharmaceuticals may provide beneficial health effects. The dissolution of articles and materials in direct and prolonged contact with human skin is often estimated using artificial SSFL and *in vitro* test methods as a surrogate for *in vivo* measurements. Only a limited number of artificial SSFLs include sebum constituents. Mounting evidence suggests that the chemical composition of an artificial SSFL is important for accurate *in vitro* modeling of potential dissolution on human skin. For example, gold readily dissolved in artificial SSFL that contained certain sulfur-containing amino acids (Brown et al., 1982; Rapson, 1982, 1985), but not in artificial SSFL that lacked these constituents (Lidén et al., 1998a). Copper dissolution decreased as the sodium chloride concentration of artificial SSFL increased (Boman et al., 1983). Additionally, the amount of dissolution from a test article in an individual constituent of artificial SSFL can be less than the amount of dissolution in the SSFL mixture (Collins, 1957; Hemingway and Molokhia, 1987; Stauber et al., 1994).

The role of individual artificial SSFL constituents in the dissolution of metals, coupled with the observation that dissolution levels in individual constituents can differ from that of the mixture, highlight the need for a comprehensive artificial SSFL and *in vitro* test system that accurately match SSFL *in vivo* (Flint, 1999). A comprehensive artificial SSFL and *in vitro* dissolution test system could provide accurate estimates of material bioaccessibility (amount of material that is available for absorption when dissolved *in vitro* using a cell-free surrogate of a biological fluid) (Ellickson et al., 2001), which in turn would improve estimation of material bioavailability (the rate and extent of absorption of dissolved material) (Zartarian et al., 2005). A better understanding of material bioavailability would ultimately improve the quality of data available for risk decision making to protect even the most susceptible persons from adverse health effects due to chemicals that leach from articles in contact with the skin.

The purposes of this article are to: (1) review the measured composition of human SSFL, (2) review published formulations of artificial SSFL, and (3) describe available test methodologies for investigating dissolution *in vitro*. Finally, we present opportunities for standardizing a new

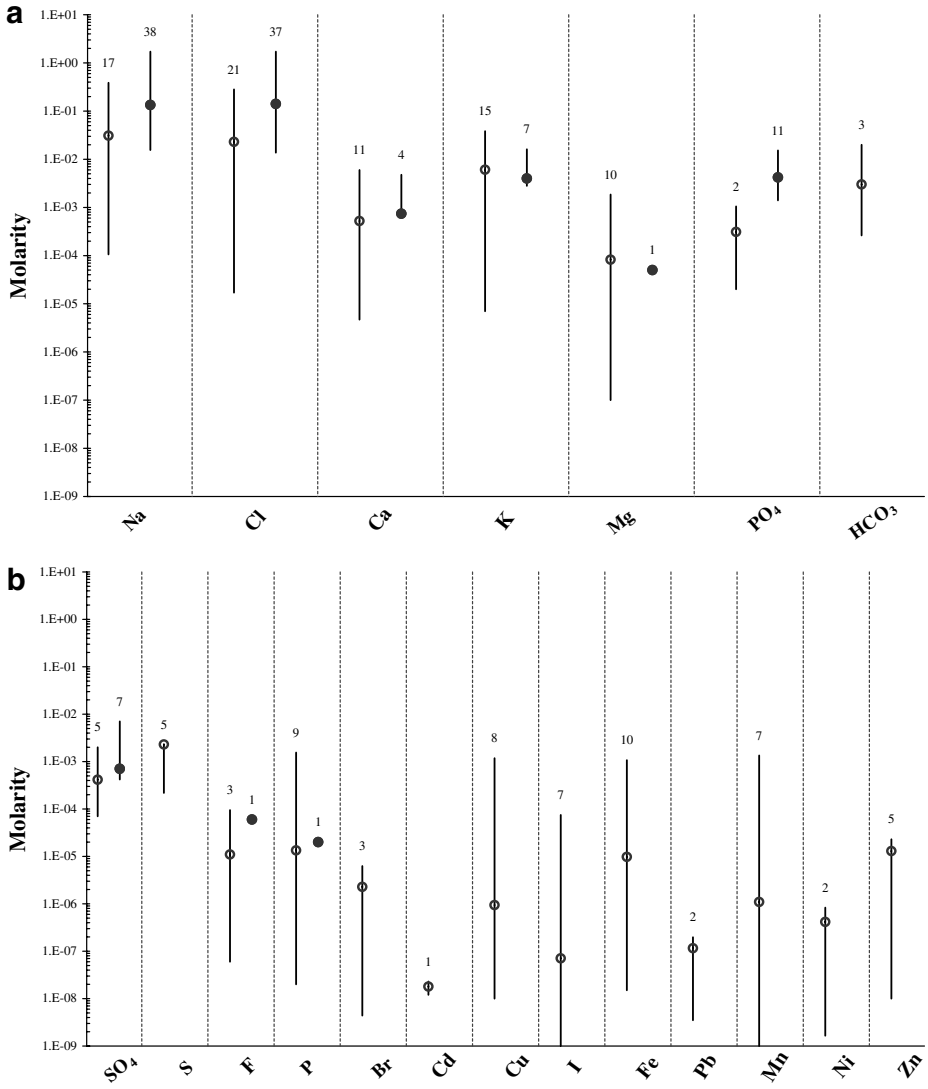

Fig. 1. Composition of adult human non-exercise-induced eccrine sweat and artificial SSFL: (a) electrolytes, (b) ionic constituents, (c) organic acids and carbohydrates, (d) amino acids, (e) nitrogenous substances, and (f) vitamins and miscellaneous constituents. The circle in each line (open = human, shaded = artificial) is the median and the bottom and top of each line are the minimum and maximum, respectively. The number above each line is the number of literature sources reporting values for that constituent of human sweat or the number of formulations of artificial SSFL. Constituent values for human sweat are aggregate data from multiple literature sources so the minimum or maximum may be an individual measurement or an average value. (d) Ala = alanine, Arg = arginine, Asp = aspartic acid, Cit = citrulline, Glu = glutamic acid, Gly = glycine, His = histidine, Ile = isoleucine, Lys = lysine, Orn = ornithine, Phe = phenylalanine, Thr = threonine, Trp = tryptophan, Tyr = tyrosine, and Val = valine. (f) Thia = thiamine (B1), Ribo = riboflavin (B2), Nia = niacin (B3), Pant = pantothenic acid (B5), Pyr = pyroxidine (B6), Fol = folic acid (B9), Asc = ascorbic acid (C), DHA = dehydroascorbic acid, Ino = inositol, Chol = choline, and PABA = *p*-aminobenzoic acid.

comprehensive model of artificial SSFL and *in vitro* test dissolution methodologies.

2. Composition of human skin surface film liquids (SSFL) and artificial SSFL

Human SSFL is a mixture of approximately 50% sweat and 50% sebum (Buckley and Lewis, 1960). Sweat is secreted by sweat glands, tubular structures consisting of two parts: a coil portion and a duct portion. The coiled portion is located in the dermis of the skin and functions to secrete a precursor sweat solution. The duct portion is located in the epidermis of the skin and functions to modify the precursor sweat solution by reabsorbing ionic constituents (mostly sodium and chloride) prior to the solution reaching the skin surface, where it is secreted as sweat. Thus, sweat secreted onto skin is the modified product of an active secretory function of epithelial cells that line the coiled portion of the sweat gland (Goldsmith, 1999; Guyton and Hall, 2000; Agache and Candas, 2004). Sweat on skin surface is a mixture of sweat constituents excreted by sweat glands and constituents formed as by-products of the skin surface maturation and desquamation processes and metabolism by skin bacteria.

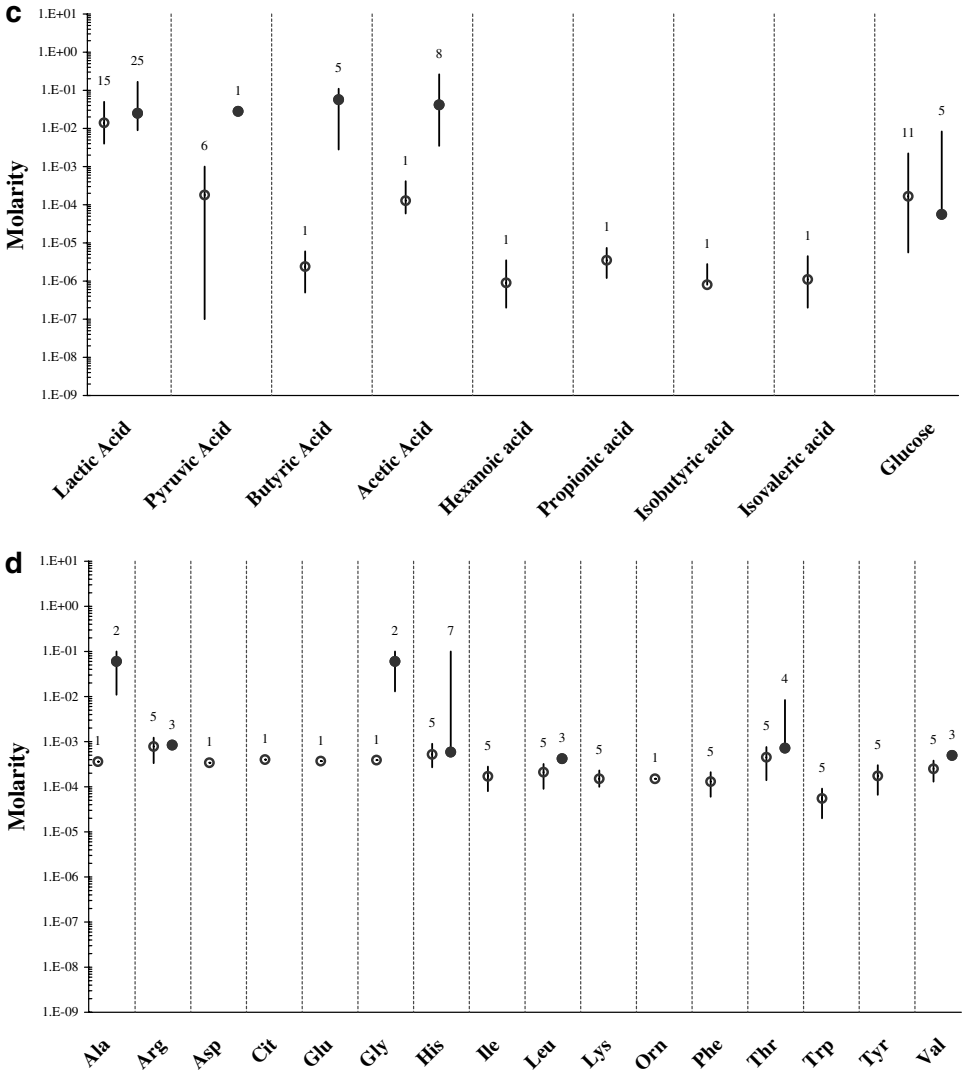

Fig. 1 (*continued*)

Sebum is secreted by pilosebaceous units, structures consisting of a sebaceous gland physically connected via a sebaceous duct to a hair follicle (Leyden, 1995; Agache, 2004). Sebaceous glands are present in all areas of the skin except for the palms of the hands and soles of the feet (Zouboulis, 2004). The sebaceous gland consists of undifferentiated, differentiated, and mature sebocyte cells. Undifferentiated sebocyte cells, located at the periphery of a sebaceous gland, are pushed through the maturation zone in the gland, where they are filled with a freshly synthesized lipid mixture to develop into differentiated sebocyte cells. These cells then pass through the zone of necrosis in the gland where they increase 100- to 150-fold in volume by accumulating more lipids to form mature sebocyte cells. This increased volume due to lipid accumulation causes the mature sebocyte cells to disintegrate, which results in the release of sebum into the sebaceous duct. Sebum is then secreted via hair follicles onto the surface of the skin within approximately eight days of mature sebocyte cell disintegration. The sebum found on skin surfaces is a mixture of sebum secreted by the sebaceous glands and epidermally derived lipids (Rothman, 1954; Schmid and Chelf, 1976; Leyden, 1995; Agache, 2004; Pragst et al., 2004).

2.1. Sweat

In this review, the term human sweat is defined as non-exercise-induced eccrine (thermoregulatory) sweat secreted by healthy adult humans. Apocrine sweat is beyond the scope of this review. The composition of sweat can vary depending upon: the person; body region (Buckley and Lewis, 1960); age; diet; season; degree of acclimation; activity level (Shirreffs and Maughan, 1997; Patterson et al., 2000, 2002; Hayden et al., 2004; Morgan et al., 2004); gender (Shirreffs and Maughan, 1997; Jacobi et al., 2005); race; and sampling technique (Robinson and Robinson, 1954).

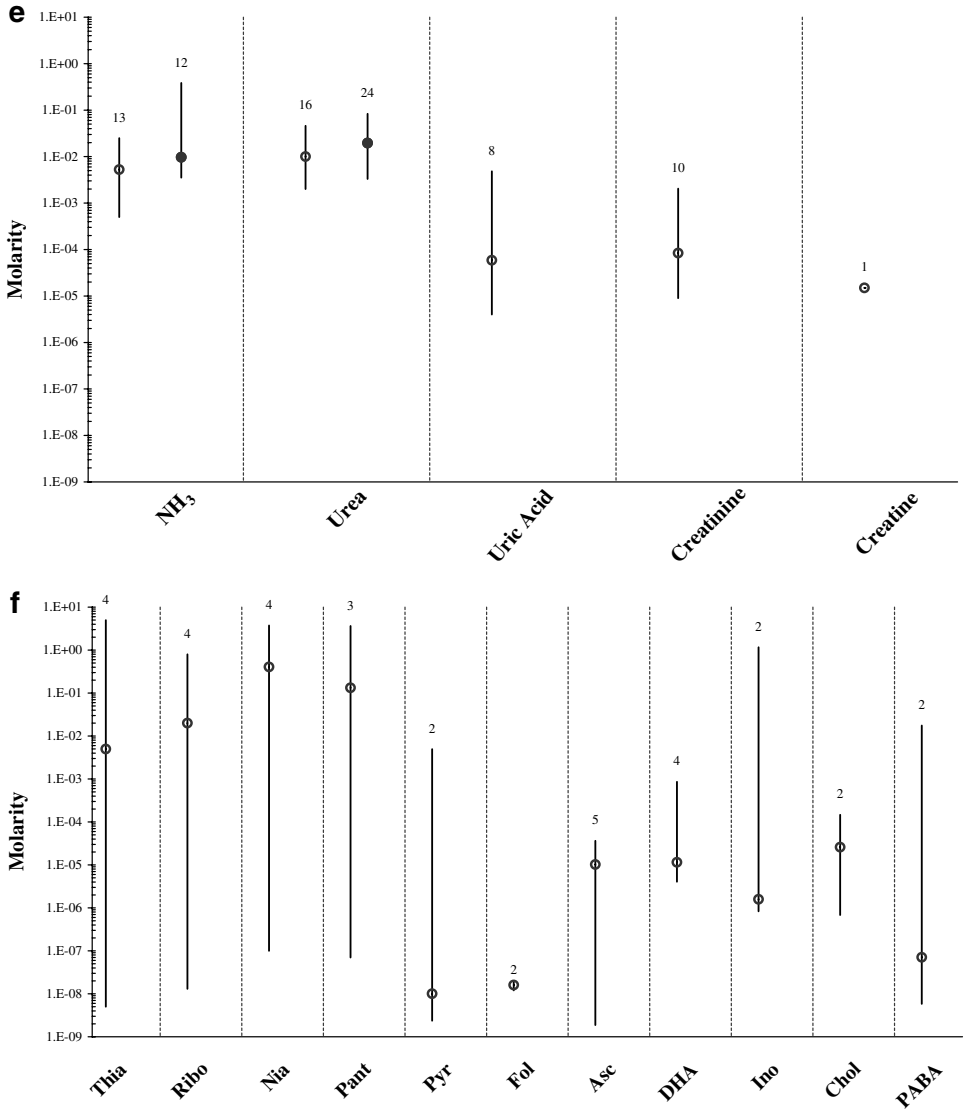

Fig. 1 (*continued*)

Human sweat is composed of highly variable amounts of primary electrolytes, ionic constituents, organic acids and carbohydrates, amino acids, nitrogenous substances, and vitamins and miscellaneous constituents (Fig. 1). Sweat is 99.0–99.5% water and 0.5–1.0% solids (half inorganic and half organic), with specific gravity of 1.001–1.008 (Robinson and Robinson, 1954; Rothman, 1954; Spector, 1956; Geigy, 1970, 1981; Altman and Dittmer, 1974; Agache and Candas, 2004).

Artificial SSFL formulations are used as *in vitro* surrogates of human sweat. The term artificial SSFL is used throughout this review and encompasses historically used terms such as "artificial sweat," "acidic artificial sweat," "artificial perspiration," "synthetic perspiration," "synthetic sweat," "sweat simulant," and "simulated sweat." Table 1 summarizes the compositions of 45 different formulations of artificial sweat identified by a search of literature published from January 1940 to December 2005. Because the dissolution of a material was often influenced by the concentration of artificial SSFL constituents, we defined a formulation as a recipe with a unique combination of constituent concentrations. For example, Chiba et al. (1997) used 0.15, 0.17, 0.51, 0.86, and 1.71 M sodium chloride solutions to model artificial SSFL, and for the purposes of this review, each solution is considered a unique formulation.

2.1.1. Electrolytes

The reported concentrations of the primary electrolytes measured in human sweat (Fig. 1a) include: sodium (Na), chloride (Cl), calcium (Ca), potassium (K), magnesium (Mg), phosphate (PO_4), and hydrogen carbonate (HCO_3) (Chopra et al., 1940; Mickelsen and Keys, 1943; Darling, 1948; Locke et al., 1951; Robinson and Robinson, 1954; Rothman, 1954; Kuno, 1956; Spector, 1956; Elze and Oelsner, 1957b; Brusilow and Gordes, 1964; Reed, 1969; Geigy, 1970; Altman and Dittmer, 1974; Kaiser et al., 1974; Stüttgen and Schaefer, 1974; Snyder et al., 1975; Iyengar et al.,

Table 1
Compositions of 45 artificial SSFL historically used to investigate dissolution *in vitro*

Formulation constituents	References
Na, Cl, K, lactic acid, NH_3, urea	Altkofer et al. (2005)
Na, Cl, lactate, NH_3, urea	Cheng et al. (2005)
Na, Cl, PO_4, histidine	Schimper and Bechtold (2005)
Na, Cl, K, lactic acid, urea	Ariza et al. (2004)
Na, Cl, lactic acid, acetic acid, NH_3, urea	Fonseca et al. (2004)[a]
Na, Cl, K, lactate, urea	Cairns et al. (2004)
Na, Cl, lactic acid, alanine, glycine, methionine, serine, threonine, urea	Hansen et al. (2003), Van Lierde et al. (2005)
Na, Cl, Ca, K, Mg, F, P	Sutton and Burastero (2003)
Na, PO_4, triolein, Tween 85®	Sartorelli et al. (1999)[g]
Na, Cl	Chiba et al. (1997)[b], Mawn et al. (2005)
Na, Cl, SO_4, lactic acid, urea	Haudrechy et al. (1993, 1994, 1997)
Na, Cl, PO_4, lactic acid, histidine	Nelson et al. (1993)[g]
Na, Cl, SO_4, lactic acid, urea, triolein, sodium oleate, tristearin, sodium stearate	Hemingway and Molokhia (1987)
Na, Cl, Ca, K, PO_4, SO_4, lactic acid, glucose, arginine, histidine, leucine, threonine, valine, NH_3, urea, OH	Boman et al. (1983)[c]
Cysteine	Brown et al. (1982)
Glutathione	Brown et al. (1982)
D-penicillamine	Brown et al. (1982)
Alanine	Brown et al. (1982)
Histidine	Brown et al. (1982)
Histidine, H_2O_2	Brown et al. (1982)
Glycine, H_2O_2	Brown et al. (1982)
Na, Cl, lactic acid, acetic acid, NH_3, urea	ISO (1982, 2003), Randin (1987a,b, 1988), Wainman et al. (1994)[a]
Na, Cl, lactic acid, urea	Pedersen et al. (1974), Menné and Solgaard (1979), Fischer et al. (1984), Menné et al. (1987), Emmett et al. (1988, 1994), Knudsen et al. (1993), Kanerva et al. (1994), Knudsen and Menné (1996), Nygren and Wahlberg (1998), Flint et al. (1998), Lidén et al. (1998a,b), CEN (1998), Lidén and Carter (2001), Nilsson et al. (2002), Jensen et al. (2003), Fenker et al. (2004), Filon et al. (2004), Abraham et al. (2005a,b)[d]
Na, Cl, SO_4, lactic acid, urea, triolein, tristearin	Fisher (1973), Williams et al. (2005)
Na, Cl, lactic acid, urea	Collins (1957)[d]
Na, Cl, lactic acid, acetic acid, butyric acid, pyruvic acid, NH_3, urea	Elze and Oelsner (1957a), Randin (1987a,b)
Na, Cl, PO_4, lactate, butyric acid, urea, harlotan, saturated alcoholic egg lecithin, Russian tallow	Roddy and Lollar (1955)[e]
Na, Cl, acetic acid, butyric acid	Eisler and Faigen (1954)
Na, Cl, PO_4, lactic acid	Eisler and Faigen (1954)
Na, Cl, SO_4, lactic acid, urea, triolein, tristearin	Eisler and Faigen (1954)
Na, Cl, lactic acid, urea, OH	Eisler and Faigen (1954)[f]
Na, Cl, lactic acid, butyric acid, NH_3, urea	Gallay and Tapp (1941)
Na, Cl, PO_4, glucose, acetic acid, NH_3, urea	Jordinson (1941)
Na, Cl, PO_4, glucose, acetic acid, urea	Jordinson (1941)
Na, Cl, NH_3	Jordinson (1941)
Na, Cl, acetic acid	Jordinson (1941)
Na, Cl, PO_4, lactic acid, butyric acid, urea, harlotan, saturated alcoholic egg lecithin, Russian tallow	Colin-Russ (1940, 1943, 1945), Katz and Samitz (1975)[e]

[a] Concentration of acetic acid differed between Fonseca et al. (2004) and ISO (1982) formulations.
[b] Five different formulations of SSFL, each with unique concentrations of Na and Cl.
[c] Three different formulations of SSFL that differ in concentrations of Na, Cl, and OH.
[d] Concentration of lactic acid differed between Pedersen et al. (1974) and Collins (1957) formulations.
[e] Concentrations of Na and lactate (ionized lactic acid) differed between Roddy and Lollar (1955) and Colin-Russ (1940) formulations.
[f] Two different formulations of SSFL, each with unique concentrations of Na, Cl, lactic acid, and urea.
[g] Artificial SSFL that was not used for dissolution studies.

1978; Stauber and Florence, 1988; Goldsmith, 1999; Guyton and Hall, 2000; Agache and Candas, 2004; Highsmith et al., 2005). In general, concentrations of electrolytes in sweat were highly variable and may span several orders of magnitude. Electrolyte constituents were mostly Na and Cl, with lesser amounts of Ca, K, and PO_4 (Robinson and Robinson, 1954; Rothman, 1954).

The concentrations of primary electrolytes in the 45 formulations of artificial SSFL were generally within ranges reported for human sweat; however, median value were

not always equal to human sweat. Most formulations lacked many of the electrolytes present in human sweat (Fig. 1a). For example, some are as minimal as a solution of Na and Cl ions (Chiba et al., 1997; Mawn et al., 2005). Among the 45 formulations of artificial SSFL, 7 lacked Na, 8 lacked Cl, 41 lacked Ca, 38 lacked K, 44 lacked Mg, 34 lacked PO_4, and all lacked HCO_3 (Table 1).

2.1.2. Ionic constituents

Human sweat is reported to contain highly variable amounts of ions (Fig. 1b), including sulfate (SO_4), sulfur (S), fluorine (F), phosphorous (P), bromine (Br), cadmium (Cd), copper (Cu), iodine (I), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), and zinc (Zn) (Chopra et al., 1940; Robinson and Robinson, 1954; Rothman, 1954; Kuno, 1956; Spector, 1956; Reed, 1969; Geigy, 1970, 1981; Altman and Dittmer, 1974; Stüttgen and Schaefer, 1974; Snyder et al., 1975; Iyengar et al., 1978; Stauber and Florence, 1988). Among these constituents, SO_4 and S were most abundant, with very low, but quantifiable levels of the metal ions present in human sweat.

Although some formulations of artificial SSFL included SO_4, F, and P at concentrations within ranges reported for human SSFL, none included S, Br, Cd, Cu, I, Fe, Pb, Mn, Ni, and Zn (Fig. 1b).

2.1.3. Organic acids and carbohydrates

The measured concentrations of organic acids and carbohydrates reported in human sweat are summarized in Fig. 1c (Mickelsen and Keys, 1943; Robinson and Robinson, 1954; Rothman, 1954; Kuno, 1956; Spector, 1956; Elze and Oelsner, 1957b; Reed, 1969; Geigy, 1970, 1981; Altman and Dittmer, 1974; Kaiser et al., 1974; Stüttgen and Schaefer, 1974; Goldsmith, 1999; Guyton and Hall, 2000; Agache and Candas, 2004). Lactic acid was the most abundant of these constituents and, on average, constitutes 0.28% of sweat (Spector, 1956; Altman and Dittmer, 1974). The high concentrations of both lactic acid and pyruvic acid contribute to the acidity of SSFL (Agache and Candas, 2004).

The concentrations of organic acids and carbohydrates in 45 artificial SSFL formulations are also summarized in Fig. 1c. The concentration of lactic acid and glucose in artificial SSFL formulations were generally within ranges reported for human SSFL. The median concentrations of pyruvic acid, butyric acid, and acetic acid in artificial SSFL were two orders of magnitude higher than in human sweat (Table 1).

2.1.4. Amino acids

The reported concentrations of 16 amino acids in human sweat: alanine, arginine, aspartic acid, citrulline, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, ornithine, phenylalanine, threonine, tryptophan, tyrosine, and valine are summarized in Fig. 1d (Hier et al., 1946; Spector, 1956; Reed, 1969; Altman and Dittmer, 1974; Stüttgen and Schaefer, 1974). Cystine (oxidized cysteine), methionine, proline, and serine were also reported constituents of human sweat, but quantitative concentration data are lacking (Itoh and Nakayama, 1952; Rothman, 1954; Elze and Oelsner, 1957a; Geigy, 1970).

The concentrations of amino acids, when included in formulations of artificial SSFL, are summarized in Fig. 1d. The median level of arginine was similar between human and artificial SSFL, whereas the median levels of alanine and glycine in artificial SSFL exceeded reported values in human sweat by two orders of magnitude. The median values of histidine and threonine in human and artificial SSFL were similar, but the maximum values in artificial SSFL were notably greater. The median levels of leucine and valine artificial SSFL exceeded the maximum level of human SSFL. Among the 45 formulations of artificial sweat, only four formulations (three by Boman et al., 1983, and one by Hansen et al., 2003) included more than one of the amino acids reported in human sweat. All artificial SSFL formulations lacked 9 of the human SSFL amino acids: aspartic acid, citrulline, glutamic acid, isoleucine, lysine, ornithine, phenylalanine, tryptophan, and tyrosine. Other artificial formulations utilized amino acids not present in human sweat, *i.e.*, penicillamine (Brown et al., 1982) or those present at unknown concentrations in human sweat, *i.e.*, cysteine, methionine, and serine (Hansen et al., 2003; Van Lierde et al., 2005).

2.1.5. Nitrogenous substances

The measured concentrations of nitrogenous constituents reported in human sweat are summarized in Fig. 1e, and included ammonia (NH_3), urea, uric acid, creatinine, and creatine (Chopra et al., 1940; Mickelsen and Keys, 1943; Robinson and Robinson, 1954; Rothman, 1954; Kuno, 1956; Spector, 1956; Elze and Oelsner, 1957a; Brusilow and Gordes, 1964; Reed, 1969; Geigy, 1970, 1981; Altman and Dittmer, 1974; Stüttgen and Schaefer, 1974; Goldsmith, 1999; Guyton and Hall, 2000; Agache and Candas, 2004).

Among the nitrogenous constituents in human sweat, only NH_3 and urea were included in formulations of artificial SSFL. Median concentrations of NH_3 and urea were similar to human eccrine sweat (Fig. 1e).

2.1.6. Vitamins and miscellaneous constituents

The highly variable concentrations of vitamin constituents measured in human sweat are summarized in Fig. 1f, and included: thiamine (B1), riboflavin (B2), nicotinic acid or niacin (B3), pantothenic acid (B5), pyridoxine (B6) and pyridoxal or folic acid (B9), ascorbic acid (C) and/or its oxidation product dehydroascorbic acid, inositol, choline, and *p*-aminobenzoic acid (Mickelsen and Keys, 1943; Robinson and Robinson, 1954; Rothman, 1954; Kuno, 1956; Reed, 1969; Altman and Dittmer, 1974; Stüttgen and Schaefer, 1974). Biotin (vitamin H) may be present in trace amounts (Spector, 1956). Phenol, concentration range 2×10^{-3} to 9×10^{-4} M (Reed, 1969; Altman and Dittmer, 1974) and histamine, concentration range 9×10^{-8} to

2×10^{-7} M (Altman and Dittmer, 1974; Geigy, 1981) were also reported constituents of human sweat. Among the 45 formulations of artificial SSFL, none included any of these vitamins or miscellaneous constituents (Fig. 1f).

2.1.7. Oxidizers

The oxygen content of human sweat may vary from saturated levels in thin films of sweat to oxygen deficient in crevices formed between the skin and articles such as watchcases, buttons, clips, earrings, etc. (Morgan and Flint, 1989). Additionally, nitric oxide (NO), an oxidative free radical, has been measured on the skin surface (mean concentration = 3.4 μM). Skin surface NO production may be the result of bacterial conversion of nitrate in sweat to nitrite, followed by reduction of nitrite to NO and water by low skin pH (Weller et al., 1996). Since oxidizers like NO release oxygen when they decompose, metabolism of sweat nitrate could be a source of sweat oxygen.

The standard method EN 1811:1998: Reference test method for release of nickel from products intended to come into direct and prolonged contact with the skin (European Committee for Standardisation (CEN), 1998) uses artificial SSFL saturated with air.

2.1.8. pH

Measured values of pH reported for whole body sweat (except as noted) are summarized in Table 2 (median = 5.3). Values of whole body sweat in Table 2 span six orders of magnitude, from a very acidic pH 2.1 (Haudrechy et al., 1997) to an alkaline pH 8.2 (Altman and Dittmer, 1974). Sweat pH may vary during a period of sweating, *i.e.*, pH may become less acidic or more acidic (Robinson and Robinson, 1954) and by body region (Collins, 1957).

The pH of artificial SSFL formulations ranged from 1.2 to 9.5 (Brown et al., 1982). To assess the effects of alkaline sweat, investigators (Jordinson, 1941; Collins, 1957; Brown et al., 1982; Emmett et al., 1988, 1994; Wainman et al., 1994; Schimper and Bechtold, 2005) used pH > 7. Given such wide variability in sweat pH, many investigators have studied dissolution of a test article over a range of pH values (Gallay and Tapp, 1941; Collins, 1957; Brown et al., 1982; Hemingway and Molokhia, 1987; Emmett et al., 1988, 1994; Haudrechy et al., 1993, 1994, 1997; Wainman et al., 1994; Abraham et al., 2005b; Schimper and Bechtold, 2005).

2.1.9. Summary

Human eccrine sweat is composed of electrolytes, ionic constituents, organic acids and carbohydrates, amino acids, nitrogenous substances, and vitamins. It contains variable amounts of NO and oxygen and has a median pH of 5.3. Historic and current SSFL formulations vary in sweat constituent identity and concentration, pH, and oxygen content under which they are used to estimate chemical dissolution on human skin.

Table 2
Values of whole body eccrine sweat pH for adult humans[a]

Reference	Minimum	Average ± Std. dev.	Maximum
Abraham et al. (2005b)	5.8	7.0 ± 0.5	7.5
Jacobi et al. (2005)[b]		5.6 ± 0.4	
Jacobi et al. (2005)[c]		4.3 ± 0.4	
Agache (2004)[d]	4.0	4.9	5.6
Agache (2004)	4.5	5.2	5.9
Haudrechy et al. (1997)	2.1	5.3 ± 1.3	6.9
Boman et al. (1983)		5.1	
Geigy (1981)		5.8 ± 0.7	
Kaiser et al. (1974)	5.0		
Altman and Dittmer (1974)	3.8		8.2
Geigy (1970)	4.0		6.8
Reed (1969)	4.2		7.5
Buckley and Lewis (1960)[e]	4.5		5.0
Collins (1957)[e]	5.3	5.9 ± 0.3	6.5
Elze and Oelsner (1957a)	3.8		5.6
Spector (1956)	3.8		6.5
Robinson and Robinson (1954)	4.0		6.8
Rothman (1954)[f]	3.8		5.6
Rothman (1954)[g]		5.7	
Rothman (1954)[g]		6.5	
Rothman (1954)[g]	4.0		6.0
Chopra et al. (1940)	4.6	5.1 ± 0.4	5.9

[a] pH values are aggregate data from multiple literature sources so the minimum or maximum may be an individual measurement or an average value.
[b] Value for women.
[c] Value for men.
[d] Face sweat.
[e] Palmar sweat.
[f] Rothman (1954, p. 221).
[g] Rothman (1954, p. 222).

2.2. Sebum

Human sebum is reported to be a mixture of non-polar lipids including squalene, wax esters, triglycerides, and free fatty acids, with lesser amounts of cholesterol esters and free cholesterol (Fig. 2) (Rothman, 1954; Schmid and Hunter, 1971; Schmid and Steiner, 1975; Schmid and Chelf, 1976; Thody and Shuster, 1989; Abrams et al., 1993). At human skin surface temperature, sebum has a specific gravity of 0.91–0.93 and exists in both solid and liquid phase (Rothman, 1954; Leyden, 1995; Motwani et al., 2001; Agache, 2004). Sebum spreads itself on the skin surface as a relatively thin, unevenly distributed sheet that ranges in thickness from <0.05 μm in sebum-poor areas to >4 μm in sebum-rich areas such as the face (Sheu et al., 1999). Sebum secretion rates vary among facial topographical regions (Youn et al., 2005a), with climatic season (Agache, 2004; Youn et al., 2005b), and with age (Zouboulis, 2004). The composition of sebum is relatively constant for each person but varies markedly between persons (Rothman, 1954; Downing et al., 1969; Green et al., 1984; Nordstrom et al., 1986a; Agache, 2004).

Among the 45 formulations of artificial SSFL summarized in Table 1, only five included an artificial sebum component. A brief search of the dermatology, personal care,

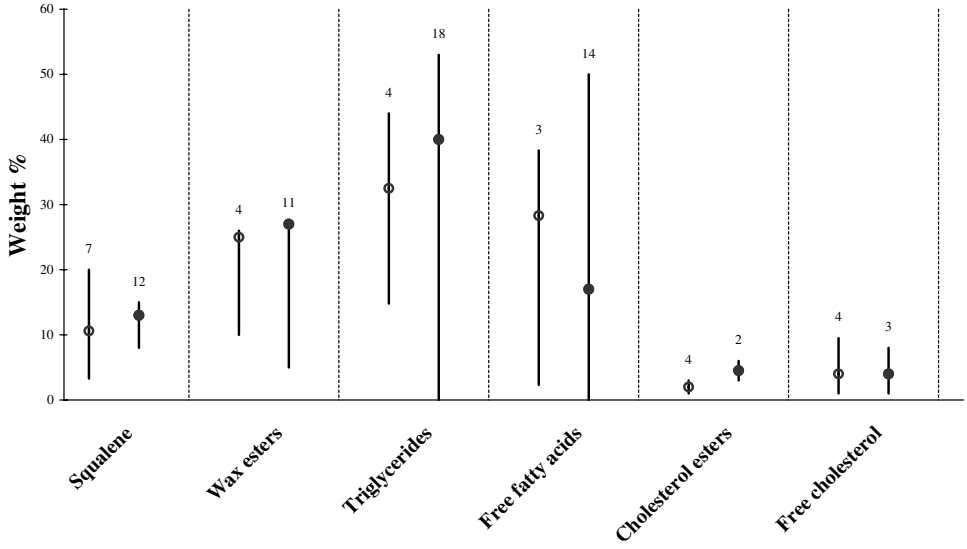

Fig. 2. Composition of adult human sebum and artificial SSFL and sebum. The circle in each line (open = human, shaded = artificial) is the median and the bottom and top of each line are the minimum and maximum, respectively. The number above each line is the number of literature sources reporting values for that constituent of human sebum or the number of formulations of artificial SSFL. Constituent values for human sebum are aggregate data from multiple literature sources so the minimum or maximum may be an individual measurement or an average value.

laundry, and cosmetic literature identified 13 additional formulations of artificial sebum (for a total of 18 formulations); these latter 13 formulations were not used in dissolution studies (Table 3).

2.2.1. Squalene

Reported values of the squalene content of human sebum are summarized in Fig. 2. Squalene is a 30-carbon hexaene compound and a major constituent of human sebum (Rothman, 1954; Spector, 1956; Reed, 1969; Downing et al., 1969; Nicolaides, 1974; Strauss et al., 1976; Abrams et al., 1993; Leyden, 1995; Agache, 2004; Pragst et al., 2004; Zouboulis, 2004). The median reported measurement is 10.6%, with a range of 3.3% (Spector, 1956) to 20% by weight (Pragst et al., 2004). According to calculations by Strauss et al. (1976), the theoretical squalene content of human sebum is 12.0%.

The squalene content of artificial sebum formulations are also summarized in Fig. 2. Among the 18 artificial sebum formulations, six (including all five SSFL formulations used in dissolution studies) lacked squalene. For the remaining 12 formulations that included squalene, the median (13% by weight) was higher than the median reported value and the theoretical content of human sebum, but the range was consistent (Blanc et al., 1989; Nelson et al., 1993; Motwani et al., 2001, 2002; Agache, 2004; Musial and Kubis, 2004).

2.2.2. Wax esters

Measured values of the wax ester content of human sebum are summarized in Fig. 2 (Downing et al., 1969; Nicolaides, 1974; Strauss et al., 1976; Leyden, 1995; Agache, 2004; Pragst et al., 2004; Zouboulis, 2004). The long-chain fatty alcohols of sebaceous wax esters have

Table 3
Compositions of 18 artificial sebum formulations

Formulation constituents	References
Triglcerides, free fatty acids	Katsuta et al. (2005)
Squalene, wax esters, triglycerides, free fatty acids, cholesterol esters, free cholesterol	Agache (2004)
Squalene, wax esters, triglycerides, free fatty acids, free cholesterol	Musial and Kubis (2004)
Squalene, wax esters, triglycerides, free fatty acids	Motwani et al. (2001, 2002)[a]
Triglycerides	Sartorelli et al. (1999)[b]
Squalene, triglycerides, free fatty acids, free cholesterol, octadecanol	Nelson et al. (1993)
Squalene, wax esters, triglycerides, free fatty acids, cholesterol esters	Blanc et al. (1989)
Triglycerides, free fatty acids	Hemingway and Molokhia (1987)[b]
Triglycerides	Fisher (1973), Williams et al. (2005)[b]
Triglycerides	Eisler and Faigen (1954)[b]
Triglycerides, harlotan, saturated alcoholic egg lecithin	Colin-Russ (1940, 1943, 1945), Roddy and Lollar (1955), Katz and Samitz (1975)[b]

[a] Eight different formulations of artificial sebum, each with unique levels of unsaturated and saturated wax esters, triglycerides, and free fatty acids.
[b] Sebum lipid classes represented in artificial SSFL used for dissolution studies.

similar unsaturated structure (*i.e.*, types of carbon chain branching, and double bond location at an even number of carbons from the carboxyl group) to those of the fatty acids. Wax ester carbon chains that are saturated (reduced to alcohols) are subjected to extensive elongation, with the end result being a predominant chain length of 20 carbons (Stewart, 1992). The median reported measurement of wax ester content in human sebum was 25%, with a range of 10% (Spector, 1956) to 26% (Agache, 2004). Strauss et al. (1976) estimate that the theoretical (unsaturated and saturated) wax ester content of human sebum is 26.0%.

Among 11 artificial sebum formulations that included wax esters (none of which were used in dissolution studies), the median level was 27% by weight, and content ranged from 5% to 27%. This median slightly exceeded the reported value and theoretical content of human sebum, and the range more than spanned values found in human sebum (Fig. 2). In the cosmetics industry, oleyl oleate is used to mimic unsaturated wax esters, whereas myristyl myristate, palmityl palmitate, palmitic acid mytistyl ester, and stearyl stearate is used to mimic 14–18-carbon chain length saturated wax esters (Motwani et al., 2001, 2002). Other investigators have used lanolin (sheep sebum with paraffin wax and water added) to mimic both unsaturated and saturated wax esters in artificial human sebum (Blanc et al., 1989; Musial and Kubis, 2004). Human sebum is composed of wax monoesters, which are a minor component of lanolin.

2.2.3. Triglycerides

Triglycerides (neutral fats) are a major lipid constituent class of pure sebum secreted via hair follicles onto the surface of the skin (Fig. 2) (Rothman, 1954; Spector, 1956; Downing et al., 1969; Reed, 1969; Nicolaides, 1974; Strauss et al., 1976; Stewart, 1992; Leyden, 1995; Motwani et al., 2001; Agache, 2004; Pragst et al., 2004; Zouboulis, 2004). Pure human sebum triglycerides have unsaturated and saturated components (Motwani et al., 2001) mixed at a ratio of 1:2 (Nordstrom et al., 1986b). On the skin surface, bacterial lipases hydrolyze certain triglycerides to small amounts of mono- and diglycerides and free fatty acids (Stewart, 1992; Leyden, 1995; Motwani et al., 2001; Agache, 2004; Zouboulis, 2004). The median reported value of the triglyceride content of human sebum was 32.5%, with a range of 14.8% to 44% (Reed, 1969).

Among the 18 artificial sebum formulations, the median triglyceride level (40%) was higher and the range was larger (range = 0.02% to 53% by weight) than values reported in human sebum (Fig. 2). For the 13 artificial sebum formulations that were not used in dissolution studies, the median (43%) exceeded the median human value and the range (10% to 53%) spanned human sebum values. For the five SSFL artificial formulations used in dissolution studies, triglyceride levels (<0.4%) were far below normal human sebum values. Early investigators (Colin-Russ, 1940, 1943, 1945; Roddy and Lollar, 1955; Katz and Samitz, 1975) used tallow (animal fat) to mimic unsaturated and saturated triglycerides in human sebum. More recently, to mimic unsaturated triglycerides, researchers used triolein, trimyristolein, or tripalmitolein (Eisler and Faigen, 1954; Fisher, 1973; Hemingway and Molokhia, 1987; Nelson et al., 1993; Sartorelli et al., 1999; Motwani et al., 2001, 2002; Agache, 2004; Katsuta et al., 2005) but some still used material of animal origin (Musial and Kubis, 2004). To mimic saturated triglycerides, researchers used trimyristin (14-carbon chain length), tripalmitin (16-carbon chain length), or tristearin (18-carbon chain length) (Eisler and Faigen, 1954; Fisher, 1973; Hemingway and Molokhia, 1987; Nelson et al., 1993; Motwani et al., 2001, 2002).

2.2.4. Free fatty acids

Measured values of the free fatty acid content of human sebum are summarized in Fig. 2. Free fatty acids are formed on the skin surface by bacterial enzymatic hydrolysis of triglycerides and are thought to be responsible in part for skin's naturally low pH (Rothman, 1954; Freinkel and Shen, 1969; Agache, 2004). These free fatty acids have unusual structural features (Stewart, 1992; Leyden, 1995). For example, some skin surface-derived fatty acids have a 16-carbon chain length for both saturated and unsaturated fatty acids rather than an 18-carbon chain length, which differentiates them from the fatty acids synthesized in other human tissues or obtained from diet (Stewart, 1992). The major free fatty acids in sebum were either 14-, 16-, or 18-carbon length chains (Haahti et al., 1962; Motwani et al., 2001) with sapienic acid (16-carbon chain) being the predominant species. Approximately 21 different lipids constitute 87.55% of fatty acids on skin, with the remaining 12.45% composed of over 200 different fatty acids. Thirty-seven percent of fatty acids on the human skin surface are "biologically valuable" fatty acids: palmitic (25.3%), myristic (6.9%), stearic (2.9%), oleic (1.9%), and linoleic (0.5%) (Nicolaides, 1974). The median reported value of the free fatty acid content of human sebum was 28.3%, with a range of 2.3% to 38.3% (Reed, 1969).

Among the 14 formulations of artificial sebum that included free fatty acids, the median (17% by weight) was lower than reported for human sebum and the range (0.02% to 50% by weight) spanned values reported for humans (Fig. 2). Only one artificial SSFL formulation that was used in a dissolution study included free fatty acids, but at a level (0.02%) far below that reported for humans. To mimic unsaturated free fatty acids, researchers used myristoleic acid (14-carbon chain length), palmitoleic acid (16-carbon chain length), and oleic acid or sodium oleate (18-carbon chain length) (Hemingway and Molokhia, 1987; Blanc et al., 1989; Nelson et al., 1993; Motwani et al., 2001, 2002; Katsuta et al., 2005). Palmitoleic acid is common in mice, but rare in humans; in lieu of palmitoleic acid, human sebum contains sapienic acid (Katsuta et al., 2005). According to Collins (1957), on an equal mass concentration basis, oleic acid caused just 5% of the corrosion of steel observed in a solution of sodium chloride. To mimic saturated free fatty acids, researchers used lauric acid (12-carbon chain length), myristic acid (14-carbon

chain length), palmitic acid (16-carbon chain length), and stearic acid or sodium stearate (18-carbon chain length) (Hemingway and Molokhia, 1987; Blanc et al., 1989; Nelson et al., 1993; Motwani et al., 2001, 2002; Agache, 2004; Musial and Kubis, 2004; Katsuta et al., 2005).

2.2.5. Cholesterol esters

Cholesterol esters are reported to be a minor constituent of human sebum (Fig. 2) (Nicolaides, 1974; Leyden, 1995; Agache, 2004; Pragst et al., 2004; Zouboulis, 2004). Cholesterol and cholesterol esters in normal human SSFL are derived from skin cell degeneration (Downing et al., 1987), sebaceous gland production, and esterification of cholesterol to cholesterol esters by skin bacteria (Puhvel, 1975). The median reported value for cholesterol esters was 2%, with a range of <1% (Nicolaides, 1974) to 3% (Agache, 2004; Pragst et al., 2004). Calculations by Strauss et al. (1976) suggest that the theoretical cholesterol ester content of sebum is 3.0%.

Among the 18 formulations of artificial sebum, only two (Blanc et al., 1989; Agache, 2004) included cholesterol esters, but the median level (4.5%) exceeded human sebum (Fig. 2); these formulations were not used for dissolution studies.

2.2.6. Free cholesterol

Free cholesterol is derived on the skin surface (Downing et al., 1987) and is a minor, but variable, constituent of human sebum (Fig. 2) (Rothman, 1954; Spector, 1956; Haahti et al., 1962; Reed, 1969; Leyden, 1995; Pragst et al., 2004; Zouboulis, 2004). Using gas chromatography analysis, Haahti et al. (1962) determined that cholesterol is a chief component among unsaponifiable materials in sebum. The median reported estimate of free cholesterol in human sebum was 4%, with a range of 1% (Leyden, 1995) to 9.5% (Reed, 1969). Theoretically, the free cholesterol content of sebum was 1.5% (Strauss et al., 1976).

Only three of the 18 formulations of artificial sebum included cholesterol (Nelson et al., 1993; Agache, 2004; Musial and Kubis, 2004). The median level (4%) and the range (1% to 8% by weight) of these formulations were consistent with human sebum (Fig. 2). These three artificial sebum formulations were not used for dissolution studies.

2.2.7. Summary

Human sebum is composed of squalene, wax esters, triglycerides, free fatty acids, cholesterol esters, and free cholesterol. Historic and current SSFL formulations and artificial sebum vary in sebum constituent identity and relative proportion of constituents. For studies that aimed to estimate chemical dissolution on human skin, only triglycerides and free fatty acids were included in the artificial SSFL, but at levels well below human sebum.

3. *In vitro* dissolution test methodologies

A variety of *in vitro* test systems were available for assessing dissolution in artificial SSFL, including a static system (Kanapilly et al., 1973) and its variations (CEN, 1998; ISO, 2003), flow-through systems (Ansoborlo et al., 1999), and electrochemical systems (Randin, 1987a,b, 1988; Haudrechy et al., 1993, 1994, 1997).

Several considerations are potentially important in the design of a dissolution study, including the accuracy and precision of an analytical method used to quantify leachate, duration of the planned test (*i.e.*, contact time), temperature of artificial SSFL, composition of laboratory equipment used to contain artificial SSFL, and the use of agitation in the test system. Equally important in the design of a dissolution study is the accurate characterization of the physicochemical properties of the test article or material under study.

3.1. Quantitative analytical method

Use of a validated standard analytical method with known reporting limits is recommended to minimize the error associated with quantifying the mass of analyte that dissolves from a test article into SSFL. The two most common reporting limits are the limit of detection (LOD), defined as the mass of analyte that gives a mean signal three standard deviations above the mean blank signal, and limit of quantification (LOQ), defined as the mass of analyte that gives a signal ten standard deviations above the mean blank signal (Kennedy et al., 1995).

Mathematical modeling prior to undertaking a dissolution study is recommended to maximize the likelihood that the mass of analyte dissolved from a test article will exceed the analytical method LOD and LOQ. For example, in the case of a homogeneous powder material, calculation of the mass of analyte that will dissolve only requires knowledge of an analytical method reporting limit, chemical dissolution rate constant of the powder, contact time, and specific surface area (SSA) of the powder (Mercer, 1967):

$$M_D = M_0[1 - \exp(-1.04 \cdot SSA \cdot k \cdot t)] \quad (1)$$

where

M_D	mass of powder dissolved
M_0	mass of powder at time 0
SSA	specific surface area, cm^2/g
k	chemical dissolution rate constant, $g/(cm^2\ day)$
t	time (days)

Normalizing the calculated value of M_D for the volume of SSFL in the *in vitro* dissolution test system provides an estimate of concentration to compare with the LOD or LOQ of a given analytical method. Adjusting M_0 to ensure that the dissolved analyte mass exceeds the appropriate reporting limit by a factor of three or more may be prudent. Alternatively, Eq. (1) can be rearranged to solve for M_0 by substituting M_D for the appropriate LOD or LOQ (multiplied by a safety factor). Some standard methods require that the volume of SSFL completely covers the test article at a ratio of 1 mL SSFL per cm^2 of test article

surface area (CEN, 1998; ISO, 2001), whereas other methods require that the volume of SSFL be a depth of about 10 mm in the container used for the *in vitro* dissolution test (ISO, 2003). For test articles and materials with biologically relevant internal and external surface areas, it is prudent to use an adequate volume to expose both the internal and external surfaces to artificial SSFL (Altkofer et al., 2005).

For poorly soluble test articles and materials, complete digestion of the test material to its dissolved form during preparation for analysis is necessary to accurately and precisely quantify the initial or remaining mass of undissolved analyte using spectroscopy. Because many spectroscopy methods are validated with soluble metal salt standard reference materials, the ability of a method to fully quantify the initial or remaining undissolved analyte mass may not be fully known for poorly soluble materials (Stefaniak et al., 2005).

3.2. Time

Time is both a practical consideration and a biological consideration in the design of *in vitro* dissolution studies. Among the *in vitro* dissolution studies reviewed (see Table 1), durations ranged from 1 hour (Jordinson, 1941; Nygren and Wahlberg, 1998; Abraham et al., 2005b) to 120 days (Chiba et al., 1997). From a practical standpoint, an *in vitro* dissolution test should be of sufficient length to ensure that the amount of material that dissolves exceeds the applicable analytical method reporting limit. The duration and number of time points of an *in vitro* dissolution test should also be sufficient to adequately characterize the dissolution behavior of a material. Dissolution may be single-phase (linear with time), biphasic (rapid initial phase followed by slower long-term phase), or multi-phasic (rapid initial phase, slower long-term phase, and a third very long-term phase). Note that the time required for biphasic or multi-phase dissolution could be several weeks to months, depending on the characteristics of the test article.

From a biological standpoint, the time that a test article will reasonably remain in contact with the skin is an important factor in the study design. For example, if the test article is a disposable latex glove or a fastener such as a button or zipper on clothing, then direct and continuous contact might be limited to a fraction of a day, whereas if the test piece is an article of jewelry such as a ring, contact time with the skin could be on the order of weeks to months or years. Consideration is also needed for the periodic contact and removal of articles from the human SSFL environment. Drying, oxidation, and other surface alterations of the article can influence the bioaccessibility of chemicals. Thus, the determination of the intrinsic dissolution behavior of an article in an artificial SSFL may not provide all information needed to assess actual human exposure to chemicals from the article during actual use. Finally, it most *in vitro* dissolution tests are accelerated artificial methods which can aid in predicting the dissolution of an article over the long-term. These short-term tests, however, should not replace long-term studies (Randin, 1987a,b).

3.3. Artificial SSFL temperature

Variability due to external and internal factors and to regional body differences makes it difficult to define a "normal" temperature for the human skin surface (Rothman, 1954; Agache, 2004). The skin surface is cooler than blood (37 °C) (Agache, 2004). The median skin surface temperature calculated from values reported for resting adult humans is 36.3 °C with range of 32.7 to 45.0 °C (Locke et al., 1951; Spector, 1956; Menné and Solgaard, 1979; Healy and Copland, 2000; Agache, 2004; Okamoto-Mizuno et al., 2005).

The temperature of artificial SSFL among *in vitro* dissolution studies ranged from 10 °C (Hemingway and Molokhia, 1987) to 100 °C (Colin-Russ, 1940, 1943). Depending on the test article of interest, artificial SSFL temperature may influence dissolution. For example, dissolution of sulfate and chromic oxide from leather increased as artificial SSFL temperature increased (Colin-Russ, 1940, 1943). Similarly, deterioration of leather samples in artificial SSFL increased as temperature increased (Roddy and Lollar, 1955). Hemingway and Molokhia (1987) report that the rate of nickel dissolution increased by a factor of 2.5 for every 10 °C rise in temperature between 10 and 40 °C (at constant pH). According to Menné and Solgaard (1979), dissolution of some nickel alloys was sensitive to temperature change (20 *versus* 35 °C), whereas for other nickel alloys dissolution was more sensitive to solvent composition (artificial SSFL *versus* distilled water).

3.4. Container material composition

To ensure that laboratory equipment does not influence observed dissolution, the material composition of the *in vitro* dissolution test container should be carefully chosen. For example, slowly dissolving hydrolyzable polyvalent metallic materials may "plate" onto the walls of polypropylene containers, thereby potentially biasing estimates of the mass dissolved; citrate or other chelators help to minimize the loss of dissolved materials to the container wall (Kanapilly et al., 1973). A range of container materials were used for *in vitro* dissolution studies, including polycarbonate plastic (Katz and Samitz, 1975; Samitz and Katz, 1975), polyethylene plastic (Lidén et al., 1998a), polypropylene plastic (CEN, 1998; Mawn et al., 2005), polytetrafluoroethylene plastic (CEN, 1998), polystyrene plastic (CEN, 1998), glass of unspecified material composition (Hemingway and Molokhia, 1987; Emmett et al., 1988; Chiba et al., 1997; CEN, 1998), borosilicate glass (ISO, 2001), and Pyrex® (Randin, 1987a,b, 1988; ISO, 2003).

Practical considerations for selecting a container material include thermal capacity (ISO, 2003), resistance to acids (CEN, 1998), and test article specific considerations. For dissolution studies of nickel in artificial SSFL, Method EN 1811 (CEN, 1998) recommends use of a non-metallic,

nickel-free container material, pre-treated with nitric acid. To fully aerate artificial SSFL for investigating dissolution of metals, a variety of container configurations such as three-necked round bottom flasks or three-compartment Pyrex cells are available (Hemingway and Molokhia, 1987; Randin, 1987a,b, 1988). Regardless of material type and configuration, all dissolution test system containers should be covered to prevent evaporation of SSFL and subsequent precipitation of artificial SSFL constituents or leachate. Covering a container also maintains a constant relative humidity (RH) in the dissolution test system; the corrosion of some metal specimens increases as RH increases (Collins, 1957; Lind, 1972). The material composition of the container used for storing samples after collection and until analysis should meet all of the same criteria as the material composition of the container used for the *in vitro* dissolution test.

3.5. Agitation

In vitro dissolution tests can be performed with or without relative motion between the sample and artificial SSFL. In dissolution systems with motion, artificial SSFL is agitated to increase contact of the test article with SSFL and presumably to simulate real-world motion of the test article when in contact with human skin. In dissolution systems without motion, both the test article and the artificial SSFL remain static throughout the test. Investigators have used a variety of agitation techniques, including shaking using a mechanical shaker (Abraham et al., 2005a,b) or water bath (Nilsson et al., 2002; Emmett et al., 1988, 1994; Altkofer et al., 2005) or wrist action shaker (Mawn et al., 2005), stirring using a magnetic stirrer (Randin, 1987a,b, 1988), ultrasonic agitation (Nygren and Wahlberg, 1998), and rotating (Jensen et al., 2003).

3.6. Test article characterization

Characterization of the physicochemical properties of a test article is critical for understanding the underlying mechanisms of dissolution and potential mechanisms of toxic action of bioaccessible material. Analytical techniques most often used to characterize the physicochemical properties of test articles included scanning electron microscopy, Rutherford backscattering spectrometry, atomic force microscopy, and optical microscopy to evaluate morphology (Ariza et al., 2004; Fenker et al., 2004; Fonseca et al., 2004) and energy dispersive X-ray spectrometry to identify elemental constituents (Menné et al., 1987; Lidén et al., 1998b). With regard to measurement of the initial mass of analyte in a test article, some investigators used spectroscopy (Samitz and Gross, 1960; Emmett et al., 1988; Lidén et al., 1998a; Nygren and Wahlberg, 1998), whereas others relied on manufacturer-provided compositional data (Pedersen et al., 1974; Randin, 1987a,b, 1988; Haudrechy et al., 1993, 1994, 1997; Chiba et al., 1997; Flint et al., 1998; Lidén and Carter, 2001; Jensen et al., 2003).

4. Opportunities for estimation of dissolution of test articles in artificial SSFL

In general, the concentrations of individual constituents, pH, and temperature of formulations of artificial SSFL are not always within ranges reported for human SSFL. Additionally, most artificial formulations lack many constituents present in human SSFL. Thus, both a need and opportunity exist for standardizing the composition of artificial SSFL and test methodologies for investigating dissolution *in vitro*. Identifying a minimum artificial SSFL composition and dissolution test methodology for bioaccessibility screening presents a significant challenge. Table 4 summarizes our recommendations for the essential and desirable components of artificial SSFL and *in vitro* dissolution test methodologies. It is our intention that these recommendations form the basis of a dialogue for harmonization of the composition of artificial SSFL and for *in vitro* dissolution test methodologies.

4.1. Composition and formulation of artificial SSFL

To provide results that can be used to protect even the most susceptible persons from adverse health effects due to chemicals that leach from articles in direct and prolonged contact with the skin, a comprehensive artificial SSFL formulation should accurately match the *in vivo* composition of human sweat and sebum. A key opportunity for standardizing artificial SSFL is defining a SSFL with constituents and constituent levels consistent with those in human SSFL. Given the potential importance of diverse sweat constituents in dissolution, a comprehensive artificial SSFL formulation should be used unless, or until, sufficient data exist for that test article to justify exclusion of the constituent class from the artificial SSFL formulation. The opportunity to standardize artificial SSFL is straight forward because levels of constituents in human SSFL are independent of the test article. Inclusion or exclusion of a constituent class from artificial SSFL will be more difficult to implement because dissolution is test article-dependent.

4.1.1. Sweat

With regard to defining an artificial SSFL consistent with human sweat constituents, the SSFLs developed by Boman et al. (1983) are the most comprehensive models identified in this review (see Table 1). We recommend the Boman et al. (1983) formulations as the basis for the sweat component of a new comprehensive artificial SSFL. To guide formulation of the Boman et al. SSFLs into a comprehensive artificial SSFL, the median values (in units of M) of individual constituents of human sweat are:

- Electrolytes: Na (3.1×10^{-2}), Cl (2.3×10^{-2}), Ca (5.2×10^{-3}), K (6.1×10^{-3}), Mg (8.2×10^{-5}), PO_4 (3.1×10^{-4}), and HCO_3 (3×10^{-3});
- ionic constituents: SO_4 (4.2×10^{-4}), S (2.3×10^{-3}), F (1.1×10^{-5}), P (1.3×10^{-5}), Br (2.3×10^{-6}), Cd

Table 4
Recommendations for measuring dissolution of test articles in direct and prolonged contact with skin surface film liquids (SSFL)

Test system parameter	Recommendation[a]
Artificial SSFL composition	
Sweat	
Electrolytes	E?
Ionic constituents	E?
Organic acids and carbohydrates	E?
Amino acids	E?
Nitrogenous substances	E?
Vitamins and miscellaneous substances	E?
Oxidizers	E?
pH	E?
Sebum	
Squalene	E?
Wax esters	E?
Triglycerides	E?
Free fatty acids	E?
Cholesterol esters	E?
Free cholesterol	E?
In vitro dissolution test methodologies	
Analytical method	D
Time	D
Artificial SSFL temperature	D
Container material composition	D
Agitation	D
Test article characterization	
Pre-characterization	D
Post-characterization	O

[a] ? denotes that not all SSFL constituents or constituent classes may be pertinent in the dissolution of a test article; E = essential information with the understanding that not all constituents or constituent classes may be pertinent in the dissolution of a specific test article. Justification of the exclusion of any constituents should be documented; D = desired information that is important and should be documented, but that is not essential; O = optional information that would be valuable.

(1.8×10^{-8}), Cu (9.4×10^{-7}), I (7.1×10^{-8}), Fe (9.8×10^{-6}), Pb (1.2×10^{-7}), Mn (1.1×10^{-6}), Ni (4.2×10^{-7}), and Zn (1.3×10^{-5});

- organic acids and carbohydrates: lactic acid (1.4×10^{-2}), pyruvic acid (1.8×10^{-4}), butyric acid (2.4×10^{-6}), acetic acid (1.3×10^{-4}), hexanoic acid (9.0×10^{-7}), propionic acid (3.5×10^{-6}), isobutyric acid (8.0×10^{-7}), isovaleric acid (1.1×10^{-6}), and glucose (1.7×10^{-4});
- amino acids: alanine (3.6×10^{-4}), arginine (7.8×10^{-4}), aspartic acid (3.4×10^{-4}), citrulline (4.0×10^{-4}), glutamic acid (3.7×10^{-4}), glycine (3.9×10^{-4}), histidine (5.2×10^{-4}), isoleucine (1.7×10^{-4}), lucine (2.1×10^{-4}), lysine (1.5×10^{-4}), ornithine (1.5×10^{-4}), phenylalanine (1.3×10^{-4}), threonine (4.5×10^{-4}), tryptophan (5.5×10^{-5}), tyrosine (1.7×10^{-4}), and valine (2.5×10^{-4});
- nitrogenous substances: NH_3 (5.2×10^{-3}), urea (1.0×10^{-2}), uric acid (5.9×10^{-5}), creatinine (8.4×10^{-5}), and creatine (1.5×10^{-5});
- vitamins: thiamine (5.0×10^{-3}), riboflavin (2.0×10^{-2}), niacin (4.1×10^{-1}), pantothenic acid (1.3×10^{-1}), pyridoxine (1.0×10^{-8}), folic acid (1.6×10^{-8}), ascorbic acid (1.0×10^{-5}) and/or its oxidation product dehydroascorbic acid (1.1×10^{-5}), inositol (1.6×10^{-6}), choline (2.6×10^{-5}), and *p*-aminobenzoic acid (7.1×10^{-8});
- oxidizers (oxygen-saturated SSFL); and
- pH (similar to median human value of 5.3; additional values in range 2.1–8.2).

4.1.2. Sebum

The formulation developed by Musial and Kubis (2004) contained the most lipid classes with constituents at levels consistent with human sebum (see Table 3 and Fig. 2) and is recommended as the basis for the sebum component of a new comprehensive artificial SSFL. To develop this formulation into a new comprehensive artificial sebum more representative of human sebum:

- squalene: adjust to a level that is consistent with the median reported (10.6%) in human sebum,
- wax esters: augment lanolin to raise the wax ester content to a level that is more representative of the median reported (25%) in human sebum,
- triglycerides: maintain 33% level consistent with that of human sebum; augment the animal-derived material to include triglycerides more representative of human sebum (see for example, Motwani et al., 2001),
- free fatty acids: raise the fatty acid content to a level that is more representative of the median (28.3%) reported value for human sebum and include unsaturated constituents (see for example, Motwani et al., 2001),
- cholesterol esters: add cholesterol esters to a level that is more representative of the median (2%) reported in human sebum,
- free cholesterol: maintain 4% level consistent with that of human sebum.

The exact mechanism by which sweat and sebum combine to form SSFL on human skin is not fully known, precluding definitive guidance on an optimum methodology for formulating artificial SSFL *in vitro*. For example, Colin-Russ (1945) reported preparing artificial SSFL by adding 500 mL of artificial SSFL at 35 °C to a molten mixture of sebum (harlotan, saturated alcoholic egg lecithin, and tallow) and using a powerful whisk. In contrast, Hemingway and Molokhia (1987) were able to emulsify glycerol trioleate, sodium oleate, and sodium stearate in artificial SSFL, whereas glycerol tristearate could not be dispersed despite warming and agitation (vigorous stirring and ultrasonic agitation). As such, the authors reported skimming glycerol tristearate from the artificial SSFL surface, which resulted in a SSFL with a "slightly opaque emulsified look." An alternative to emulsification is dissolving artificial sebum in a non-polar solvent, *e.g.*, 3:1 chloroform–methanol (Motwani et al., 2001), 2:1 chloroform–methanol (Schmid and Hunter, 1971; Schmid, 1973; Schmid et al., 1973a; Schmid and Chelf, 1976; Abrams et al., 1993; Agache, 2004), or 3:2 toluene–ethanol (Schmid et al., 1973b) co-solvent mixture, then evaporating the solvent in the test system container to form a sebum layer on the container. Upon forming a sebum layer in the container, the test article could be placed on top of the sebum layer followed by immersion in artificial SSFL.

In summary, for a given test article or material, a comprehensive formulation of artificial SSFL should be used to estimate dissolution until sufficient test data exist to justify exclusion of a SSFL constituent or constituent class. For this reason, our recommendation in Table 4 is shown as "Essential?", where ? denotes that not all SSFL constituents or constituent classes may be pertinent in the dissolution of a test article. Simply testing the effect of a single constituent on dissolution of a test article may not be sufficient because the amount of material that dissolves can be less than in the mixture of artificial SSFL (Collins, 1957; Hemingway and Molokhia, 1987; Stauber et al., 1994). To provide guidance in formulating artificial SSFL, specific examples of SSFL-constituent influences on material dissolution are summarized in Appendix A.

4.2. In vitro dissolution test methodologies

Several key opportunities for standardization of *in vitro* dissolution test methodologies exist, including considerations for the analytical method used to quantify dissolved analyte, duration of the dissolution test, artificial SSFL temperature, test system container material composition, use of agitation in the test system, and characterization of the physicochemical properties of the test article under study.

Hemingway and Molokhia (1987) recognized the potential variability in SSFL and skin conditions among persons and measured dissolution over a range of variable conditions (*e.g.*, SSFL composition, pH, and temperature). By systematically varying their test system, Hemingway and Molokhia (1987) were able to identify the most pertinent test system conditions in the dissolution of nickel. We recommend their experimental approach as a framework for future *in vitro* dissolution testing and have designated most of these *in vitro* dissolution test methodology parameters (Table 4) as "Desired." Specific examples of desired information for reporting dissolution of test articles in artificial SSFL are summarized below to guide the design of *in vitro* dissolution test systems:

- Analytical method: use a validated standard analytical method whenever available, provide information on method recovery to demonstrate accuracy and precision, and when biologically relevant, methods capable of determination of valence of ions in leachate should be used, *e.g.*, chromium(VI) *versus* chromium(III) or total chromium (Samitz and Gross, 1960; Nygren and Wahlberg, 1998; Van Lierde et al., 2005) and dithiocarbamates in natural rubber latex (Abraham et al., 2005a,b).
- Time: use mathematical modeling to ensure that dissolution results exceed analytical method detection limits. Measurements of material contact time with skin, when available, may help to estimate biologically relevant study duration. This is of special concern for material or particles that may remain on the skin after removal of an article from the skin, or after cessation of contact with a source of material or particles. For example, sequential hand washing (Linnainmaa and Kiilunen, 1994) or hand wiping (Que Hee et al., 1985) is not sufficient to completely remove cobalt or lead contamination from skin, suggesting that study durations of hours to days may be justifiable for these metals. Repeated contact of a test article with skin (*e.g.*, daily use of multiple pairs of disposable latex gloves) should also be considered.
- Artificial SSFL temperature: human skin temperature ranges from 32.7 to 45.0 °C (median = 36.3 °C), but may increase during exertion. This range of human skin temperature may also be useful for Arrhenius equation approaches which use temperature as a variable and provide improved understanding of dissolution kinetics.
- Container material composition: select an appropriate container material to minimize the potential for study bias due to loss of leachate to dissolution and storage container walls. (See for example Rosias et al., 2004; for the importance of container selection for exhaled breath condensate monitoring.)
- Agitation: the biological relevance of ultrasonic agitation for *in vitro* dissolution test systems and the influence of agitation (*e.g.*, stirring *versus* ultrasonic agitation *versus* shaking) are unclear and in need of further research.
- Test article physicochemical characterization: considerations should be given to characterization of the bulk article and the article as used in the *in vitro* dissolution test system. Detailed methodologies and strategies for characterizing size, morphology, elemental and crystalline composition, bulk and surface chemistry, surface area and density of particulate has been described (Hoover et al., 1989; Stefaniak et al., 2003, 2004; Rouleau et al., 2005a,b). If possible, characterization of a test article after exposure to SSFL should be performed if a change in the physicochemical properties of the material is likely (*e.g.*, changes in aggregation state or bulk and surface chemistry of particles). Sequential characterization of material properties may also be needed if contact is intermittent.

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Appendix A. Known caveats of artificial SSFL formulation constituents in dissolution

In general, there is a paucity of data regarding the influence of sweat and sebum constituents in dissolution of test articles and materials in artificial SSFL. To guide future investigations of material dissolution in a comprehensive artificial SSFL, examples of artificial SSFL constituents

or constituent classes with test-article-dependent influence on dissolution are summarized here:

- Electrolytes: dissolution of copper in artificial SSFL decreased as the sodium chloride concentration SSFL increased (Boman et al., 1983). In contrast, dissolution of chromium(III) increased as sodium chloride content of artificial SSFL increased (Flint et al., 1998). Cl increased the dissolution of leather (Colin-Russ, 1940), metals (Lind, 1972; Morgan and Flint, 1989), and mild steel (Collins, 1957).
- Organic acids: lactic acid increased the dissolution of chrome from chrome-tanned leather (Gallay and Tapp, 1941; Colin-Russ, 1940, 1945), likely by complexing with the chromium(III), whereas butyric acid had negligible effect on dissolution (Gallay and Tapp, 1941; Colin-Russ, 1940, 1945; Van Lierde et al., 2005). The addition of lactic acid to sodium chloride caused more corrosion of mild steel than the same concentration of sodium chloride alone or lactic acid alone (Collins, 1957). Dissolution of nickel in pure lactic acid was only 2% to 5% of lactic acid in artificial SSFL (Hemingway and Molokhia, 1987). Butyric and pyruvic acids had negligible effect on dissolution of stainless steels and nickel (Randin, 1987a,b).
- Amino acids: individual amino acids, especially sulfur-containing amino acids (*i.e.*, cysteine) in the presence of oxygen, may be needed for dissolution of gold (Rapson, 1982; Möller et al., 1999; Lidén and Nordenadler, 1999; Flint, 1999). Although amino acids in artificial SSFL can complex nickel (Morgan and Flint, 1989), the significance of alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, leucine, serine, and threonine in artificial SSFL is difficult to assess. Some of these amino acids may adsorb onto metal surfaces and act as corrosion inhibitors or complex with metal ions and thereby act as corrosion promoters (Flint, 1998). Methionine was important in the reduction of chromium(VI) to chromium(III) in chromium leached from leather (Van Lierde et al., 2005).
- Nitrogenous substances: urea had negligible effect on the dissolution of mild steel (Collins, 1957).
- Oxidizers: The presence of oxygen markedly increased the dissolution of gold-containing (Brown et al., 1982) and nickel-containing (Hemingway and Molokhia, 1987; Randin, 1988) test articles in artificial SSFL. Oxidizers may also influence valence state, and therefore, potentially toxicity. For example, formation of chromium(VI) ions from chromium(III) ions is favored in alkaline artificial SSFL that contain strong oxidizers, whereas artificial SSFL with neutral pH and oxygen content equal to atmospheric is not sufficient to oxidize chromium(III) ions (Flint et al., 1998).
- pH: dissolution of sulfate from leather (Colin-Russ, 1943), mild steel (Collins, 1957), chromium(VI) in certain soil samples (Wainman et al., 1994), and gold under certain solvent conditions (Brown et al., 1982) increased as pH increased. In contrast, dissolution of nickel from certain nickel-containing stainless steels (Haudrechy et al., 1993, 1994, 1997), wire (Hemingway and Molokhia, 1987), and earrings (Emmett et al., 1988) increased as pH decreased. For substances such as 2-mercaptobenzothiazole, dissolution increased or decreased with pH depending on the test article matrix (Emmett et al., 1994). Dissolution of zinc diethyldithiocarbamate from rubber gloves (Abraham et al., 2005b), and depending upon the artificial SSFL constituents, dissolution of nickel-plated stainless steels (Haudrechy et al., 1993, 1994), gold (Brown et al., 1982), and chromium(III) (Wainman et al., 1994) can be independent of pH.
- Free fatty acids: oleic acid (unsaturated free fatty acid) had negligible effect on dissolution of steel (Collins, 1957).

Virtually no data was available that described the role of ionic constituents, many nitrogenous substances (NH_3, uric acid, etc.), vitamins, squalene, wax esters, triglycerides, cholesterol esters, and free cholesterol in dissolution.

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